

### **ABSTRACT**

Methods are provided for detecting a mutant polynucleotide in mixture of mutant polynucleotides, wild-type polynucleotides and unrelated polynucleotides. The method uses an extension primer complementary to a first target sequence in both the wild-type and mutant polynucleotides. The method also uses a probe complementary to a second target sequence in the wild-type polynucleotides but not in the mutant polynucleotides. Extension of the primers annealed to the first target sequence in mutant polynucleotides produces long extension products. Extension of the primers annealed to the first target sequence in wild-type polynucleotides is blocked by the probe annealed to the second target sequence. Short extension products or no extension products are produced. The extension products are isolated and used in a polymerase chain reaction (PCR). The PCR preferentially amplifies long extension products.